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Results of intracutaneous tests for the detection of trichina infections in swine.

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INTRODUCTION

In a previous paper, Spindler and Cross (1939, Proc. Helminth. Soc. Wash. 6 (2): 37–42) reported the results of 5,274 intracutaneous tests for the detection of trichina infections in swine; the tests involved 55 antigens made in different ways. Data presented in that paper indicated that the antigens used were not sufficiently specific to be relied upon when tested on hogs in abattoirs.

Later Lichterman and Kleeman (1939, Amer. Jour. Pub. Health 29 (10): 1089–1102) reported the results of tests on 211 swine in which a saline extract of dried powdered larvae was used as an antigen. In these tests the condition as regards trichinosis was correctly diagnosed in 97 per cent of the hogs tested. Following publication of the above-mentioned report, saline extracts of dried powdered larvae were again tested by us in conjunction with the tests involving our own antigens. The present paper is a report of 12,435 skin tests on 1,512 swine during the period from December, 1938, to June, 1940. The report briefly summarizes (1) the results of tests with all antigens used; (2) the results of tests made with the antigen which we have found to be most efficient under practical conditions; and (3) results obtained with the saline extract antigen described by Lichterman and Kleeman (*loc. cit.*).

MATERIALS AND METHODS

In this series of tests antigens were made in a wide variety of ways including solutions of various chemical fractions of trichina larvae. The various substances used as antigens were first prepared and given a preliminary test on experimentally infected swine at Beltsville, Md. Some antigens gave unsatisfactory results and were, accordingly, discarded; others gave promising results and were then tested on both grain-fed and garbage-fed swine in slaughter houses. Methods of preparing all the individual antigens used are too extensive to recount in detail in this paper. However, one antigen composed of trichina larvae ground to a particulate condition, referred to in this paper as antigen 165, was found, in some cases, to be sufficiently superior to other antigens to warrant a brief description of the method of its preparation and a consideration of the results obtained with it.

Trichina larvae used in preparing this antigen (number 165) were obtained by peptic digestion of the flesh of experimentally infected swine. After the digestion process was completed the larvae were freed of undigested host tissue by passing the larvae through silk bolting cloth after which they were washed in 5 to 10 successive changes of 0.5 per cent sodium bicarbonate solution and then in from 5 to 10 successive changes of sterile distilled water; this was found sufficient to free the larvae of all traces of host protein. The living larvae, in approximately 10 times their own volume of sterile distilled water were then ground in a ball mill to a particulate condition. During the grinding process precautions were taken to avoid bacterial contamination. After the larvae were reduced to a particulate con-

dition, usually after 24 to 30 hours of grinding, the suspended material was allowed to settle for approximately 18 hours in the refrigerator. The material remaining in suspension was then concentrated by heating (usually below 55° C.) *in vacuo*. The residue was then further concentrated in a stream of warm air and the drying process completed over sulphuric acid *in vacuo*. The resulting dry material was then ground in an agate mortar to a particulate condition and the powder stored over calcium chloride at refrigerator temperature.

The saline extract of dried powdered larvae was prepared in strict accordance with the method outlined by Lichterman and Kleeman (*loc. cit.*).

The technic of making the tests was essentially the same as in the series already reported. The animals were restrained and 0.1 to 0.2 cc of each of the antigens being used was injected intracutaneously along the mid-line of the abdomen. This site was selected for injections because the discolorations of the skin, which may at times persist even after death of the animal, could be trimmed off and not materially affect the value of the carcass. Furthermore, it was found that reactions to tests made in skin of the abdomen are generally more distinct than those made in skin of the thigh.

In the case of antigen 165, the particulate material was suspended in the diluting fluid sometimes 24 hours before and sometimes just before testing. Diluents used were Coca's solution, physiologic saline, and more recently a freshly prepared solution of 0.5 per cent sodium chloride and 0.05 per cent sodium bicarbonate in distilled water.

Reactions to the tests were read after an interval of 15 to 20 minutes following the injections. A description of the reactions produced by antigens, as well as our interpretations of these reactions, was described in the previous report (*loc. cit.*).

One or more injections of control diluent were made simultaneously with injections of antigen on each animal tested.

All animals were slaughtered immediately after reading the results of the tests and the diaphragm of each animal was removed for digestion to determine whether it harbored trichinae.

RESULTS OF INTRACUTANEOUS TESTS

The results of the total series of 12,435 tests are summarized in table 1. It is interesting to note that 1,482 tests on infected animals (groups A and B) gave clear-cut positive reactions in only 42.78 per cent of the cases. In 21.12 per cent of these tests the reactions were classed as doubtful. In the remaining 36.09 per cent no reaction to the test antigen was apparent.

Of the 10,953 tests on uninfected swine (groups C and D) 71.15 per cent were negative, 11.33 per cent were doubtful and 17.51 per cent were positive.

The summary of the reactions produced by 2,810 injections of control diluents reveals that typical positive reactions occurred in 4.76 per cent of the cases. All positive reactions to control diluent occurred in swine in groups B and D tested in abattoirs; none occurred in swine tested at Beltsville, Md. For the entire series of control injections 7.54 per cent of the reactions were doubtful, the remaining 87.68 per cent being negative. Possible causes of non-specific reactions in swine will be discussed in a later publication.

Inasmuch as promising results were obtained by us using antigen 165, in tests on experimentally and naturally infected swine, and since the saline extract of dried larvae was reported by Lichterman and Kleeman (*loc. cit.*) to be an accurate antigen, a summary is given in table 2 of the results of our tests with each of the two antigens on unselected hogs in slaughter houses. In the case of antigen 165 distinct positive reactions were obtained in 73.25 per cent of 430 tests on hogs sub-

TABLE 1.—*Summary of results of intracutaneous tests for trichina infections in swine*

Designation and source of animals[a]	Number of swine tested	Number of tests	Reactions produced by test antigens						Number of tests	Reactions produced by control diluents					
			Positive		Doubtful		Negative			Positive		Doubtful		Negative	
			Number	Per cent	Number	Per cent	Number	Per cent		Number	Per cent	Number	Per cent	Number	Per cent
Group A (infected)	37	537	115	21.41	160	29.79	262	48.78	37	0	0.00	4	10.81	33	89.18
Group B (infected)	113	945	519	54.92	153	16.19	273	28.88	513	63	12.28	56	10.91	394	76.80
Total or average	150	1,482	634	42.78	313	21.12	535	36.09	550	63	11.43	60	10.90	427	77.63
Group C (noninfected)	18	187	18	9.62	35	18.71	134	71.65	18	0	0.00	1	5.55	17	94.44
Group D (noninfected)	1,344	10,766	1,900	17.64	1,206	11.20	7,660	71.14	2,242	71	3.16	151	6.73	2,020	90.09
Total or average	1,362	10,953	1,918	17.51	1,241	11.33	7,794	71.15	2,260	71	3.14	152	6.72	2,037	90.13
Grand total	1,512	12,435							2,810	134	4.76	212	7.54	2,464	87.68

[a] Group A, experimentally infected animals; Group B, naturally infected animals tested at slaughter houses; Group C, noninfected animals raised at Beltsville, Md.; Group D, noninfected animals tested at slaughter houses.

TABLE 2.—*Summary of results of intracutaneous tests with saline extract of dried powdered larvae and antigen No. 165 made on hogs of abattoir origin*

Antigen used	Designation of swine	No. of tests	Reactions produced by test antigen					
			Positive		Doubtful		Negative	
			Number	Per cent	Number	Per cent	Number	Per cent
Saline extract of dried larvae	infected	159	93	58.49	25	15.72	41	25.78
No. 165	do	430	315	73.25	52	12.09	63	14.65
Saline extract of dried larvae	uninfected	1,458	601	41.22	164	11.24	693	47.53
No. 165	do	3,825	805	21.04	357	9.33	2,663	69.62

sequently shown to be infected. In the case of 3,825 tests on uninfected hogs, positive reactions were obtained in 21.04 per cent (Table 2).

In the total series the presence or absence of trichinae in the hogs was correctly determined by approximately 71 per cent of the tests.

In concurrent tests on many of the same animals, using the saline extract antigen referred to, positive reactions were obtained in 58.49 per cent of those hogs later shown to be infected. In 1,458 tests on uninfected swine positive reactions occurred in 41.22 per cent (Table 2). The presence or absence of trichinae in the animal was correctly determined by approximately 53 per cent of the tests.

DISCUSSION

The results of tests herein reported are in agreement with those previously published (Spindler and Cross, *loc. cit.*). Although antigens made by a variety of ways, including chemical fractions of trichina larvae, were tested none gave reliable results when applied to swine in slaughter houses. In 11,711 tests on swine at abattoirs the status of the animals as regards trichina infection was correctly detected in approximately 63 per cent of the tests. At Beltsville, Md., however, in only approximately 46 per cent of the 724 tests conducted there, were infections correctly diagnosed. This difference is probably due to the fact that most of the tests in conjunction with the development of the various antigens were conducted at Beltsville and the results of preliminary tests with certain antigens which did not give promising results are included in that series; only those antigens that had shown promising results in preliminary tests on experimentally infected hogs were used in the abattoir series.

In the present series of tests there appears a marked increase over the previously reported series in the number of positive reactions to control injections of diluent alone. These positive reactions occurred in swine tested at one abattoir during June, 1940; the cause has not been definitely determined. Various lots of diluent were used but each contributed to the total of positive reactions noted. Samples of those diluents which gave reactions and antigens prepared with the diluents were subsequently tested on uninfected hogs at Beltsville. When used there, they uniformly failed to produce positive reactions in these animals.

SUMMARY

Results of 12,435 intracutaneous tests made with various antigens for the detection of trichina infections in swine are summarized. In the total series of 1,482 tests on infected hogs clear-cut positive reactions were obtained in only 42.78 per cent; in 36.09 per cent no reactions were apparent.

Of 10,953 tests on swine later shown to be uninfected, 71.15 per cent were negative but positive reactions occurred in 17.51 per cent.

A description is given of a method of preparing an antigen designated as number 165. This antigen is composed of bodies of trichina larvae ground to a particulate condition; this particulate material is suspended in diluent for the tests.

Results of tests with antigen 165 are compared with results of tests using a saline extract of dried powdered trichina larvae; these tests were all made on hogs in abattoirs.

In the total series of tests involving antigen 165 the condition as regards trichina infections was correctly diagnosed in approximately 71 per cent.

Using the saline extract of dried powdered larvae the condition as regards trichina infection was correctly diagnosed in approximately 53 per cent.

In the total series of injections of control diluents distinct positive reactions occurred in 4.76 per cent. All these occurred in one series of tests and the cause has not been determined.

A simple method of removing bacteria that adhere to trichina larvae. JOHN LAWRENCE AVERY, U. S. Bureau of Animal Industry.

During the course of investigations on trichinae it became desirable to separate the decapsulated larvae from bacteria and adhering debris without resorting to sterilization by chemicals. By use of the method herein described this can be accomplished at the time the larvae are freed from host tissue by peptic digestion. The method devised by the writer is purely mechanical and involves the passage of trichina larvae from the digestive fluid through a layer of sterile sand into sterile Ringer's solution; this is accomplished by gravity and the activity of the larvae.

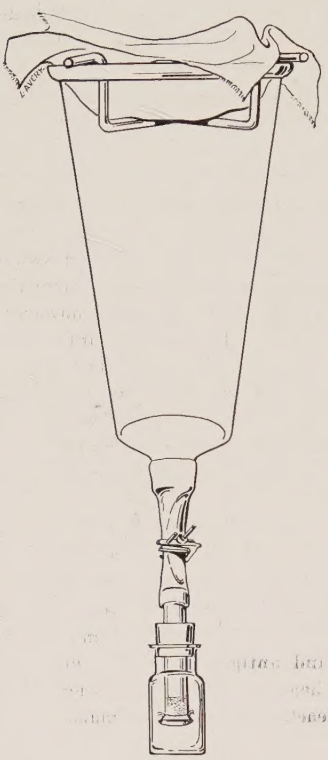

FIG. 1. An apparatus for obtaining bacteria-free trichina larvae from infected meat.

Description of the apparatus.—The portion of the apparatus used for digesting the trichinous meat is essentially the same as that described by Bozicevich (1938, U. S. Pub. Health Rpt. 53 (48): 2130–2138) and Lichterman and Kleeman (1939, Amer. Jour. Pub. Health 29 (10): 1098–1102). It consists of a 4-liter percolator with a piece of rubber tubing attached to the stem; a pinch clamp is fitted to the tube. In place of the centrifuge tube used by the above mentioned authors, a special "sand-tube" was attached to the rubber tubing.

As shown in the accompanying figure, the "sand-tube" is a test tube, 2 cm in diameter, from which the bottom has been removed. A single layer of cheesecloth was first tied over the lip of the tube and a $\frac{5}{8}$-inch layer of coarse sand placed over the cheesecloth. The tube was then inserted into a 2-ounce bottle and held in place by means of a tightly fitted rubber stopper. It was found desirable to wash the sand very thoroughly and then to boil it for several minutes in each of several changes of distilled water before setting up the apparatus; this served to remove chemical impurities that might be injurious to the larvae.

Use of the apparatus.—Before the apparatus was set up, the "sand-tube" with the collecting bottle attached was plugged with cotton and autoclaved. When cool, the stopper was removed and the bottle filled with sterile Ringer's solution to which 0.5 per cent sodium bicarbonate had been added to neutralize any acid which the decapsulated larvae might carry through from the digestive fluid. The "sand-tube" assembly was then attached to the percolator by means of the connecting rubber tubing as shown in the illustration. After the apparatus was placed in a 37° C. incubator, the pinch clamp was closed and the percolator filled with digestive fluid (7 cc hydrochloric acid, 5 grams pepsin, 1000 cc water, temperature 37° C.). The pinch clamp was then gradually opened so that the inrush of the digestive fluid would not disturb the layer of sand. Fresh trichinous meat was then ground and placed on 4 layers of cheesecloth supported by glass rods in the top of the percolator.

After 18 to 20 hours incubation, the majority of the larvae that had been freed

from host tissue had passed through the sand into the sterile modified Ringer's solution. The pinch clamp was then closed and the bottle containing the larvae detached from the sand-tube, to eliminate any chance of forcing bacteria through the sand and thereby contaminating the solution containing the larvae.

By using this apparatus it has always been possible to obtain larvae free from bacteria, if aseptic precautions had been observed throughout. Prolonged incubation of large quantities of such larvae in nutrient broth and on agar plates, under anaerobic and aerobic conditions, failed to demonstrate any contaminating bacteria.

As larvae of *Trichinella spiralis* apparently do not harbor bacteria in their alimentary tract the only problem in rendering such larvae bacteria-free is that of removing organisms adhering to the cuticle of the worm. Passage through sterile sand seems to accomplish this effectively. This method of ridding trichinae of adhering bacteria might be applied to other organisms small enough to be capable of passing through such a layer of sterile sand.

Studies on oxyuriasis. XXV. Necropsy examinations for *Enterobius vermicularis* in 72 children at Washington, D. C. MYRNA F. JONES, National Institute of Health, U. S. Public Health Service.

Examinations for the presence of *Enterobius vermicularis* were made in 72 children coming to necropsy during the years 1937 to 1939. The material in each case consisted of the intestinal tract from the duodenum to the rectum, inclusive, with the exception of one case in which the appendix and another in which the small intestine were lacking. In most cases the intestine was intact when received but in a few it had been partially opened for examination. The specimens were submitted by two local hospitals;[1] the patients had resided in the District of Columbia and nearby Maryland and Virginia.

Although we were interested in comparing the incidence of infection in such a series of cases with that based on anal swab examinations of persons comprising a similar sample of the population,[2] emphasis was placed on observations concerning the location of worms in the intestine and on attempts to recover young developing worms for study.

METHODS OF EXAMINATION

The parts of the intestine were clamped off and examined separately, i.e., the small intestine in 3 or 4 sections, the appendix, the cecum and the colon. The separate parts were slit open and examined; the contents and the scrapings from the wall were screened; and the larger worms were subsequently recovered from the screens. The series of screens used had mesh apertures ranging from 10 to 60 per inch. The fluid caught from the screening was allowed to settle in jars and was examined with the dissecting microscope for smaller forms. Scrapings from several parts of the intestinal wall of some specimens were taken before screening and examined microscopically; in a few cases portions of the small intestine were examined with the Baermann apparatus. No material was sectioned.

RESULTS OF EXAMINATIONS

E. vermicularis was found in 21 intestines or in 28 per cent of the 72 specimens examined. No worms were observed to be actually attached; none were observed in scrapings or recovered from the Baermann apparatus although most of these

[1] Children's Hospital and Gallinger Municipal Hospital supplied the specimens and certain data regarding them; their cooperation is acknowledged with grateful appreciation.

[2] Study made later at Children's Hospital to be published by Eugenia Cuvillier Jones.

latter examinations were of intestines which proved to be negative or lightly infected. The numbers of worms recovered and the regions of the intestine in which worms were found are presented in table 1.

TABLE 1.—*Number and location of Enterobius vermicularis recovered at necropsy from 21 infected children*

Degree of infection		Location in intestine		
Number worms	Number times found	Region	Number times found	
			Total	1 region only
1	3	Small intestine	6	.....
2 to 30	15	Appendix[a]	18	5
50 to 200	3	Cecum	12	1
		Colon	12	2

[a] Based on 20 intestines.

None of the cases represented a heavy infection; in only 3 of the 21 positive cases were there more than 50 worms. To what extent the number of worms may have been decreased by illness preceding death cannot be judged.

Worms were recovered less frequently from the small intestine than from the large intestine. In 3 lightly infected cases a single worm was recovered from each small intestine, whereas the greatest numbers of worms encountered in that site were in the 2 most heavily infected cases. The worms recovered from the small intestine were predominately males. In no case were worms recovered from the small intestine and not from some part of the large intestine. As indicated by the table, examination of the entire large intestine revealed a larger percentage of positive cases than would have been found by the examination of any one part. However, examination only of appendices in this series would have been a fairly reliable index of infection; in 5 of the 18 positive appendices single worms were recovered which might possibly have been missed by other methods of examination, such as examination of isolated sections or of preserved material. In 5 cases a positive finding was dependent on the examination of the appendix.

The findings analyzed according to age, race and sex are presented in table 2.

TABLE 2.—*Distribution according to age, race and sex of 72 children examined at necropsy for Enterobius vermicularis*

Age years	Number examined	Number positive	Negro		White		Male		Female	
			Number examined	Number positive	Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
0–1	34	6	21	4	13	2	14	4	20	2
2–5	19	9	12	3	7	6	9	6	10	3
6–14	19	6	15	4	4	2	10	3	9	3
Total	72	21	48	11	24	10	33	13	39	8

Obviously these groups are too small for the results to be taken as typical for such groups in general. However, these necropsy results do not differ markedly from some of the findings based on swab examinations of similar groups in the District of Columbia (Cram, 1940). Few positive cases were observed in the groups less

than 2 years of age, only about one-sixth of the group being infected. The youngest positive case was a 3-months-old white male. Pinworms were found in nearly one-half of the 2- to 5-year group and in nearly one-third of the 6- to 14-year age group, both races being included.

In comparing the two races, there was little difference in the small groups of 2- to 5-year males, 3 of 5 Negro and 3 of 4 white cases being positive. However, in other groups and in the series as a whole there were relatively fewer positive cases in the Negro than in the white race. All three of the infections of more than 50 worms were in white cases; there were 4 to 5 times as many worms recovered from the 10 white cases as were recovered from the 11 Negro cases.

Data supplied by the hospitals gave no records of known symptoms of oxyuriasis and there was no correlation of the presence of worms with the post-mortem diagnoses.

DISCUSSION

The material was disappointing in that it yielded for study few pinworms in various stages of development. Possibly some small worms may have disintegrated because of delay in examination and the considerable time involved in the examination itself. However, it is considered probable that, except for extremely heavy infections, young developing worms only a few days old would be found infrequently in cases which came to necropsy; the effect of illness on reinfection doubtless is a factor, but one which could vary with the nature of the illness.

There is considerable literature on the presence of *Enterobius* in appendices and its possible relationship to appendicitis. However, we do not know of any previous report on a series of intestinal examinations for *Enterobius* in this country, although such examinations have been made in other countries. The present findings are comparable to those of Still (1899) who reported in England on a series of 200 examinations; of the whole series, 0 to 12 years of age, 19 per cent were positive; of 100 of these, 2 to 12 years of age, 32 per cent were positive. Worms were recovered from 25 appendices of the 38 positive cases and in 6 cases worms were recovered only from the appendix. Müller (1917), in Denmark, reported on a series of necropsies including 48 cases under 15 years of age, of which 62 per cent were positive, whereas 47 per cent of the adult cases in the series were positive. Peiser (1920), in Germany, in examining a series of ceca stated that the 2- to 14-year age group was the most frequently parasitized, with 10 positive cases in the 29 cases of this age examined. More recently Chanco (1940) reported from the Philippines on the occurrence of *E. vermicularis* as revealed by necropsy examination of ceca; of a series of 200 adults, 82, or 41 per cent, were positive.

In this country infection with *E. vermicularis* undoubtedly varies with age, race and locality. Additional information based on necropsy material, even though not in itself the best index of infection, would be of considerable interest, especially if it could be correlated with swab examinations on persons in the same locality.

SUMMARY

Necropsy examination for the presence of *E. vermicularis* was made of intestines from 72 hospitalized individuals ranging from 0 to 14 years of age.

Pinworms were recovered from 21, or 28 per cent, of the intestines. The findings are discussed according to number and location of the worms in the intestine and according to the age, race and sex of the host.

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The incidence of oxyuriasis in two institutions in Puerto Rico. FREDERICK J. BRADY, National Institute of Health, U. S. Public Health Service.

The published reports on the distribution of pinworm (*Enterobius vermicularis*) infection have consistently shown high incidences in institutionalized children particularly in those surveys in which the NIH swab was employed. Surveys in the United States based on the examination of 4 or more NIH swabs on institutionalized children have shown incidences of over 70 per cent with the single exception of a 16 per cent incidence found in 63 Negro girls examined in New Orleans by Sawitz, D'Antoni, Rhude and Lob (1940, South. Med. Jour. 33(9): 913–922).

The following results are of interest because of the low incidence found in two groups of orphans in whom a high incidence was anticipated. Fifty girls in one institution and 52 boys in another, both located in Puerto Rico, were examined by 4 NIH swabs made under conditions that were optimal in view of our present knowledge. The swabs were made by trained personnel on alternate days and were used early in the morning, in most cases before the individual had been out of bed and in no case after the individual had been up more than 15 minutes.

The girls were housed in an old building with inadequate and inconvenient sanitary facilities and large crowded dormitories. The boys were housed in new buildings with good sanitary facilities and small dormitories, each containing 7 or 8 beds. All buildings had large doors and windows and nearly all dormitories of both institutions had through and through ventilation. The windows generally did not have sashes or panes but could be closed by shutters.

The following table shows the findings on the swab examinations:

Group	No.	Age range	Swab results					Total persons +	Per cent +
			4 – 0 +	3 – 1 +	2 – 2 +	1 – 3 +	0 – 4 +		
Girls ...	50	6–19	35	12	2	0	1	15	30
Boys ...	52	5–19	46	4	2	0	0	6	12

Even in this small sample of 102 persons, the number found to harbor pinworms appears significant of a low incidence of oxyuriasis. Certain factors may be operative in causing this low incidence. The groups examined were composed of persons of the white and colored race with many mulattoes. While most of the previous surveys have indicated a lower rate of infection in Negroes, in this case the number of colored persons is not enough to explain the low incidence. One or more of the following factors may be responsible for the low incidence: (1) Strong breezes blowing almost constantly through the large windows and doors, open the whole year, may sweep away many ova; (2) the shortened duration of the viability of the pinworm ova, as shown by Jones and Jacobs (in press), due to temperatures never below 62° F.; and (3) the fact that these children spent more time outdoors where the possibility of picking up viable ova was decreased.

Notes on the survival of infective horse strongyle larvae. JOHN T. LUCKER, U. S. Bureau of Animal Industry.

Recently, the writer (1938, Jour. Agr. Research 57: 335–348) pointed out (1) that infective horse strongyle larvae are known to survive prolonged exposure to low temperatures and (2) that experiments dealing with the comparison of the survival of these larvae at different temperatures which they might encounter in their environment apparently had not been reported. A preliminary experiment designed to test the influence of temperature on the life span of these larvae is described below.

About 2,000 infective horse strongyle larvae in a small quantity of water were placed on the soil in each of several small specimen bottles containing approximately equal amounts of moist sterilized sandy clay loam. The bottles were fitted with cork stoppers. One series of such preparations was placed in an incubator; a second in a cooler maintained at a temperature slightly above freezing, a third in the freezing compartment of an electric household refrigerator, and a fourth in a dark cupboard in the laboratory. At various intervals, the soil from the bottles kept in each of these situations was examined for surviving larvae by means of the Baermann apparatus with results shown in table 1.

TABLE 1.—*Comparative survival of infective horse strongyle larvae kept at different temperatures*

Bottle No.	Duration of exposure	Infective larvae recovered from soil from bottles kept at indicated mean temperatures (Centigrade)			
		31°	26°	3°	−5°
	Days	*Number*	*Number*	*Number*	*Number*
1	31	350			
2	31		1,075		
3	31			1,250	
4	31				1,050
5	66	20			
6	66		550		
7	66			685	
8	66				325
9	102	5			
10	102		3		
11	102			510	
12	102				710
13	128	1			
14	128		18		
15	128			450	
16	128				635
17	201				1,225
18	347		0		
19	347			1,050	
20	347				850

It is evident from the tabulated data that under the conditions of the experiment, infective horse strongyle larvae remained viable longer at mean temperatures of about −5° and 3° C. than at 31° C. or at variable room temperatures (mean about 26° C.). The larvae kept at room temperature did not survive as well as might have been expected on the basis of the findings of other investigators. Possibly, decomposition of organic matter in the soil, or other factors due to inadequate aeration, resulting from the fact that the bottles were stoppered, deleteriously affected the larvae kept at the higher temperatures, such influences being minimized

in the bottles kept at low temperatures. However, it is unlikely that these factors were important determinants of the general result of the experiment, because the stoppers were cork, a substance that permits exchange of gases.

Since the publication of the writer's paper, mentioned previously, Baker, Salisbury, and Britton (1939, Cornell Vet. 29: 297–308) have reported that infective horse stronglye larvae kept at room temperature, or alternately at room temperature and at 40° F., lost their reserve food material more rapidly than those kept continuously at 40° F. The results of these workers indicate a surprisingly rapid exhaustion of the reserve food material of larvae kept at room temperature. In the laboratory the larvae were subjected to light, as well as fluctuating temperatures, while those kept at 40° F. were in the dark. Hence, the authors considered their experiment as testing the relationship between activity and the life span rather than the comparative survival of the larvae at the temperatures mentioned. However, it is a known fact that temperature is an important determinant of the activity of strongyle larvae in general.

The available data, therefore, strongly suggest that infective horse strongyle larvae in a moist environment survive longer at temperatures slightly above the freezing point, or even at subfreezing temperatures, than at temperatures above 25° C.; the optimum temperature has not been determined.

Another experiment of a different nature, also related, however, to the problem of the survival of horse strongyle larvae under natural conditions in warm months of the year, was performed as follows:

Freshly passed horse feces were spread on the surface of 4 small outdoor plots (within 36″×36″ wooden frames) near Beltsville, Maryland, early in May. The plots were shaded by nearby trees during part of the day. The soil within the plots was classified as a coarse sand admixed with very small percentages of clay and humus. When sufficient time had elapsed to permit development of infective larvae, the feces on each plot were again spread out as uniformly as possible over the soil surface. At this time the feces and surface soil from a 36 square inch area of each plot were examined to determine the number of infective larvae present on this unit of the surface area. To obtain each sample, a frame 6 inches square was pressed into the ground while the feces and surface soil to a depth of about $\frac{1}{8}$ inch were removed from the inclosed area. Immediately after these samples had been collected, the feces on 3 of the plots were turned under with a spade as if a garden were being prepared. After the newly upturned soil had been raked smooth, the surface layer of another unit area was similarly removed for examination. The remaining plot was undisturbed and served as a control.

Three subsequent examinations of similar samples of the surface soil of the 3 test plots and of the feces and surface soil of the control plot were carried out. A final examination was made of the soil removed from the surface to the 1-inch level and from the 1-inch to the 2-inch level of a unit area of the control plot and of one of the 3 test plots; in taking the sample from the control plot, the feces were removed before the soil was excavated and were separately examined. The samples removed for the various examinations were from different regions of an individual plot and the samples of each series collected at one time were from unit areas comparably located in the different plots. The Baermann apparatus was used to isolate the larvae from the samples. The results of the examinations are shown in table 2.

These data (Table 2) show that turning under feces originally on the surface of the test plots left only an extremely small residuum of larvae in the newly upturned surface layer. However, in a little more than a month a considerable proportion of the buried larvae, apparently up to about 50 per cent, had reached the surface of the coarse sand. About 6 weeks later, however (about 11 weeks

TABLE 2.—*Comparative mortality of infective horse strongyle larvae on surface of and spaded under coarse sand in outdoor plots in summer season*

Date	Larvae recovered from a unit area of 36 square inches			
	Control plot	Test plots		
		Plot 1	Plot 2	Plot 3
	From feces and surface $\frac{1}{8}$ inch of soil	Before turning under (From feces and surface $\frac{1}{8}$ inch of soil)		
1938	*Number*	*Number*	*Number*	*Number*
May 27	8,600	16,100	13,450	33,000
		After turning under (From surface $\frac{1}{8}$ inch of soil)		
May 27	.....	130	30	80
July 1	24,700	8,530	2,960	17,550
August 10	2,000	28	152	55
October 6	1,400	0	1	11
	From feces			
October 12	118	.....	.....	.....
	From soil from surface level to 1-inch level			
October 12	8	24	.....	.....
	From soil from 1-inch level to 2-inch level			
October 12	1	74	.....	.....

after the larvae were buried), the surface soil of the test plots contained very few larvae. A little more than 4 months after the larvae were buried the surface soil of these plots was almost free of larvae. In the same period a marked, though less striking, diminution in the number of larvae present in the feces and surface soil of the control plot was found. Most of the surviving larvae on this plot were evidently still in the feces at the conclusion of the experiment, although some had reached the upper levels of the underlying soil. A residuum of larvae was obviously still present in the deeper soil layers of the test plot, but the data suggest that the number of survivors in the plots as a whole was small compared to the number originally developing.

The method of sampling in this experiment has obvious limitations, but from the facts at hand it is permissible to conclude that only a small fraction of infective horse strongyle larvae in feces on coarse sand soil near Beltsville, Md., survived the various environmental influences and stimuli of the summer season. Furthermore, the mortality among larvae was even greater when feces containing them were buried in coarse sand, presumably because the energy loss incident to migration through the soil rendered the larvae regaining the surface more susceptible to the influences operative there.

Taylor (1938, Vet. Rec. 50: 1265–1272) recently showed a rapid death rate of horse strongyle larvae which had ascended grass blades in outdoor experimental plots. Although the literature is replete with qualitative evidence that horse strongyle larvae can survive outdoors for long periods even under rigorous climatic conditions, the quantitative experiments of Taylor and the writer suggest that in the warm season of the year environmental factors destroy the vitality of the bulk of the larvae on an unoccupied pasture in a few months.

Some parasites newly recorded for the ruffed grouse, *Bonasa umbellus*. in the United States. Justus F. Mueller, N. Y. State College of Forestry.

While engaged in a study of parasites and disease in ruffed grouse in New Hampshire during the summer of 1940, 46 birds were examined. Search for intestinal and blood protozoans was relatively inadequate, but examination of the organs for other internal and external parasites was carried out in a thorough manner. The complete report of this study will be published in mimeograph form separately by the Fish and Game Department of New Hampshire, which agency sponsored the investigation and has given permission for the following notes to be published at this time. The following species of worms were found:

Trematodes

Leucochloridium pricei McIntosh, 1932
Harmostomum pellucidum Werby, 1928
Prosthogonimus macrorchis Macy. 1934

Cestodes

Davainea proglottina Blanchard, 1891
Hymenolepis carioca (Magalhaes, 1898) (?)
Raillietina tetragona (Molin, 1858)

Nematodes

Ascaridia bonasae Wehr, 1940
Cheilospirura spinosa Cram, 1927

Determination of the cestodes must be regarded as tentative until broad studies have been made with a view to clarifying the members of the genera involved. As yet there appears to be no general agreement as to the species of cestodes found in grouse, different authors apparently ascribing to the same form different specific determinations.

The species *Leucochloridium pricei* was first described by McIntosh (1932) from the Alaska spruce grouse, locality Alaska, and has not been reported previously from the ruffed grouse or from the United States. Trematodes which have been identified as this species were found in the bursa Fabricii, cloaca, and lower rectum of New Hampshire ruffed grouse. Heavy infections were commoner in young birds, with as many as 100 or more worms present in a single host. The parasite was one of the most frequent infections encountered, occurring in over half of all birds examined. These worms were first submitted to the Bureau of Animal Industry for examination where they were determined as *Leucochloridium* sp. by Mr. McIntosh. In a letter received from the Chief of the Zoological Division, August 15, 1940, it was stated "Your specimens differ somewhat from *L. pricei* in the distribution of the vitellaria and other minute characters." On detailed study of these worms the differences observed are not regarded by the present author as sufficient to justify a new species. My specimens are somewhat smaller than the dimensions given by McIntosh for the Alaska material, about 0.7 mm as compared to 1.17 mm. The relative size of the suckers appears to be about the same with the acetabulum in the New Hampshire specimens perhaps a little smaller than in the Alaska material. The arrangement of the ovary, testes, and uterus is very similar in the two. In some of my worms the vitellaria have slightly greater anterior-posterior limits, but in others the condition corresponds closely to that figured by McIntosh for *L. pricei*.

A single specimen of *Prosthogonimus macrorchis* was recovered from the cloaca of a male bird 233 grams in weight, from Webster, N. H. The specimen in the flattened condition measures 5.5 mm long by 3.5 mm wide. This size is somewhat smaller than that given by Macy (1934, page 10) for the type, 7.56 mm by 5.26

mm, and the uterine coils are not so abundant, but it is assumed that these differences may be due either to the younger age of my specimen or to host influence. The observed differences in other respects are no greater than those figured for *Prosthogonimus* from various hosts by Macy (1934, Plate IX). In the specimen from the grouse the oral sucker is 0.22 mm in diameter, while the ventral sucker is 0.39 by 0.35 mm. The esophagus is 0.22 mm long. The vitellaria extend to the level of the posterior edge of the testes on one side, slightly further posteriad on the other. These relationships are only slightly different from those figured for the type by Macy (1934, Plate I) and are regarded as insufficient for the establishment of a new species. *Prosthogonimus* has not previously been reported from ruffed grouse, and as pointed out by Macy in a personal communication of September 7, 1940, since the grouse is not a migratory bird it must be presumed to have received its infection locally. He goes on to state "Although I have followed the literature closely I have found no report of *Prosthogonimus macrorchis* from territory outside of the Lake States and nearby Canada. I do not know that it occurs east of Michigan, nor west of Minnesota." The present case therefore not only adds a new host to those already known for this parasite but greatly extends its range.

Previous to the present study trematodes have been regarded as rare in grouse in the United States, the only species recorded being *Harmostomum pellucidum*. The present study therefore adds two new forms. The reason for this variation in findings in New Hampshire grouse from conditions usually encountered elsewhere undoubtedly lies in the fact that the New Hampshire birds were for the most part taken on swampy or low ground where snails are abundant. In several instances these mollusks were found in their crops. In other localities collectors usually take their grouse from dry upland localities. The reason for this apparent difference of distribution of the bird in different localities is a matter of conjecture not within the province of this paper.

The general collections of this study have been deposited in the United States National Museum. Adequate numbers of *Leucochloridium* and the single specimen of *Prosthogonimus* have been included.

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Distribution of the genus *Thelandros* (Nematoda: Oxyuroidea).[1] A. C. WALTON, Knox College, Galesburg, Illinois.

The genus *Thelandros* (Wedl, 1862), with the type species, *T. alatus* Wedl 1862, is apparently confined to lizard and tortoise hosts with but one exception. Of the 18 recognized (plus 8 unnamed) species referred to this genus, all but 3 are found in various lizard hosts (representing at least 14 genera and 20 species); 2 species are reported only from tortoises (2 genera and 3 species); and 1 is reported from a Brazilian tree frog (*Hyla mesophaea*). Whether it is a matter of abundance of species or merely one of the recognition of the presence of members of the genus *Thelandros* is as yet undetermind, but up to the present 11 of the named species have been reported from African hosts while 7 (2 duplications of African

[1] Contribution from the Biological Laboratories of Knox College, No. 73.

forms) are reported from Asiatic hosts and only 2 are recorded as having come from South American hosts. Of the unnamed species referred to the genus (most of which probably belong to the known species), the majority are reported from African hosts although 2 have been found in South America and 1 in an Australian lizard (kept in the London, England, Zoo for some time). Thus far no members of the genus have been reported from European or North and Central American hosts. These regions, particularly those of Central and North America, are ones which support reptilian and amphibian faunas that are ecologically, and to some extent zoologically, equivalent to those of Africa, Asia, or South America. That it may be only a matter of time before the range of the genus *Thelandros* will be greatly extended is indicated by recent studies.

Through the courtesy of Mr. J. H. Mohr of the University of California a number of nematodes obtained from the California legless lizards *Anniella nigra* and *A. pulchra* were sent to the writer for identification. Among the specimens were a number of Oxyurid forms that undoubtedly belong to the genus *Thelandros*, thus increasing the geographical range of the genus to include the southwestern coastal area of the United States. No attempt is made to give a species designation to this *Thelandros* form since the material consisted entirely of female specimens with the exception of enough fragments of a male to enable the definite generic identification to be substantiated. Study of the genus as a whole shows that the species may be arranged into 3 groups: in one the ovarian loops are confined to the region back of the esophageo-intestinal junction (the majority of the species), in the second group the tubules extend forward into the esophageal region (2 species), and in the third group the tubules definitely make from 1 to 5 coils around the corpus of the esophagus. To this latter group belong the species *Thelandros maplestoni* (Chatterji, 1933), *T. micipsae* (Seurat, 1917), *T. seurati* (Sandground, 1936), and *T. scleratus* (Travassos, 1923). *Oxyuris* sp? of Thapar (1925), from an Australian lizard, was shifted to *Thelandros* and included in this third group by Malan (1939). The North American form, on the basis of the presence of from 3 to 6 coils of the ovarian tubules around the posterior end of the esophagus and the isthmus region of the bulb, must likewise be placed with the species of the third group. A brief description of the stout maggot-like females may assist other workers to determine the proper species designation whenever satisfactory male material becomes available.

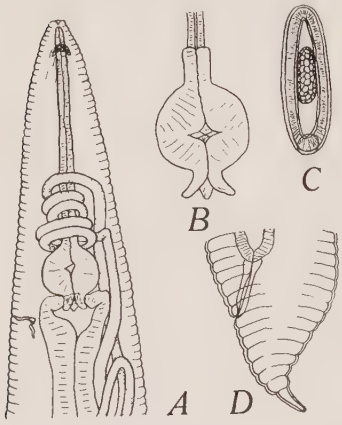

FIG. 1. *Thelandros* sp. ♀, female. A—Anterior end. B—Esophageal bulb. C—Egg. D—Posterior end.

Length, 4.75–5.25 mm; width at vulva, 0.5–0.675 mm; length of pharynx, 0.03–0.036 mm; length of esophagus, 0.7–0.72 mm; length of isthmus of bulb, 0.09–0.095 mm; bulb measurements, 0.135–0.145 mm × 0.175–0.182 mm; head-nerve ring distance, 0.068–0.072 mm; head-excretory pore distance, 1.25–1.3 mm; vulva position, median; anus-tail distance, 0.32–0.36 mm; egg measurements, 0.048–0.052 mm × 0.125–0.129 mm, with one polar plug and initial segmentation at the time of oviposition; ovarian coils prebulbar and circumesophageal.

Reference to the table shows that the genus *Thelandros* apparently is confined to terrestrial host species, and mainly to those that inhabit areas having a rela-

tively high temperature during much of the year. These hosts are lizards, tortoises, and amphibia that live in the tropical and subtropical areas of Africa, Asia, Australia, Central America, North America, and South America, and in their adjacent islands. Closely related (often identical) species are found in Europe, and search of such likely hosts will undoubtedly further extend the range of the genus *Thelandros*.

TABLE 1.—*Distribution of Thelandros species*

Species	Host		
	Lizard	Tortoise	Frog
Africa—mainly Northern Africa			
T. alatus Wedl, 1862 (= *Oxyuris uromasticola* Galeb, 1889)	*Uromastix* sp.		
T. bulbosus (Linst., 1899) Seurat, 1917	*Agama* sp. *Chalcides* sp.		
T. bulbosus annulatus Seurat, 1917	*Agama* sp.		
T. cinctus (Linst., 1897) Baylis, 1923	*Agama* sp.		
T. echinatus (Rud., 1819) Seurat, 1917	*Agama* sp. *Chalcides* sp. *Tarentola* sp.		
T. micipsae Seurat, 1917	*Chalcides* sp. *Lacerta* sp. *Scincus* sp. *Tarentola* sp.		
T. numidicus Seurat, 1918		tortoises	
T. rotundus Malan, 1939	*Agama* sp. *Pseudocordylus* sp.		
T. sahariensis Baylis, 1930	*Uromastix* sp.		
T. seurati Sandground, 1936	*Acontias* sp.		
T. sexlabiata Ortlepp, 1933		*Testudo* sp.	
T. sp.? of Baylis, 1929	*Agama* sp.		
T. sp.? of Loveridge, 1923	*Agama* sp.		
T. sp.? of Loveridge, 1923	*Agama* sp.		
T. sp.? of Loveridge, 1929	*Agama* sp.		
T. sp.? of Loveridge, 1936	*Agama* sp.		
[a] *T.* sp.? of Price	*Agama* sp.		
Asia—mainly India and Ceylon			
T. baylisi Chatterji, 1935	*Uromastix* sp.		
T. bulbosus (Linst., 1899) Seurat, 1917	*Scincus* sp.		
T. cinctus (Linst., 1897) Baylis, 1923	*Agama* sp.		
T. kasauli Chatterji, 1935	*Uromastix* sp.		
T. maplestoni (Chat., 1933) Baylis, 1936 (= *Parapharyngodon maplestoni* Chatterji, 1933; = *T. hemidactylus* Patwardhan, 1935; = *Oxyuris acanthura* of Linstow, 1904; = (?) *Oxyuris megaloon* Linstow, 1906—see Baylis, 1936)	*Calotes* sp. *Hemidactylus* sp.		
T. micruris Rauther, 1918 (= *T. alatus* Wedl of Thapar, 1925)	*Uromastix* sp.		
T. taylori Chatterji, 1935	*Uromastix* sp.		
South America—mainly Brazil			
T. oswaldocruzi Travassos, 1925			*Hyla* sp.
T. scleratus Travassos, 1923	*Tropidurus* sp.		
T. sp.? of Travassos, Freitas & Lent, 1939	*Tropidurus* sp.		
T. sp.? of T., F., & L., 1939	*Ameiva* sp.		
[a] *T.* sp.? of Chitwood	*Conolophus* sp.	(Galapagos Islands)	

TABLE 1—(*Continued*)

Species	Host		
	Lizard	Tortoise	Frog
Australian host (from English Zoo)			
T. sp.? of Malan, 1939 (= *Oxyuris* sp.? of Thapar, 1925)	*Trachysaurus* sp.		
North America			
T. sp.? (this paper)	*Anniella* spp. (U. S. A.)		
[a] *T.* sp.? of Chitwood	*Cyclura* sp. (Puerto Rico)		
[a] *T.* sp.? of Chitwood	*Leiocephalus* sp. (West Indies)		
[a] *T.* sp.? of Chitwood	(?) *Uromastix* sp. (Cent. Amer.)		

[a] These records of specimens in the Helminthological Collections of the Bureau of Animal Industry, U. S. Department of Agriculture, have been made available through the courtesy of the Chief of the Zoological Division—Dr. Benj. Schwartz.

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The finer structure of *Aplectana hamatospicula* (Nematoda).[1] A. C. WALTON, Knox College, Galesburg, Ill.

The habit of basing new species of nematodes on the presence or absence of gross anatomical structures has led to the failure in recording those details of finer nature which Chitwood and Chitwood (1937, 1938) have recently shown to be of much greater significance than is usually realized. This failure has particularly been the case in the Genus *Aplectana*. In order to close certain of these gaps some of the finer details have been worked out for several related forms—in particular for *Aplectana hamatospicula*, a recently described species found rather commonly in the Anura of the regions around the Gulf of Mexico.

The genus *Aplectana*, which recently has been removed from the Oxyuroidea, belongs to the family Cosmocercidae of the superfamily Ascaridoidea, suborder Ascaridina and order Rhabditida of the subclass Phasmidia, on the basis of general morphology (Chitwood & Chitwood, 1937). Members of the Ascaridina normally have 3 lips, one dorsal and 2 subventral in position. The inner of the two rows of cephalic papillae are always reduced. The Ascaridoidea have the outer row of these papillae well developed as opposed to the reduction or loss of some of them in the Oxyuroidea. The Cosmocercids have a distinctly valvulated esophageal bulb, an esophageal isthmus that is never subspherical, and lack a ventral precloacal sucker. Aplectanids lack even the musculature suggestive of such a structure. The paired spicules are equal, and the accessory piece or gubernaculum is normally cuticularized to some degree.

Study of profile and *en face* views of the head end of *Aplectana hamatospicula* (Fig. 1, A & B) shows the typical three-lipped condition of the group. The inner row of 6 cephalic papillae are present, although much reduced in size. The dorsal

[1] Contribution from the Biological Laboratories of Knox College, No. 74.

lip supports 2 double papillae of the outer row of cephalic structures (partially fused dorsodorsal-laterodorsals), while each subventral lip shows 1 such double papillus (partially fused ventroventral-lateroventrals) and 1 single lateral papillus in addition to the laterally placed amphidial opening. The fundamental plan of 6 cephalic nerves shows as 2 subdorsal, 2 lateral, and 2 subventral branches. The 2 amphidial nerves are entirely separate from the papillary system, being attached to the nerve collar at different points. The subdorsal and subventral nerves are each divided into 3 branches while the lateral nerves are only 2-branched. One branch goes to the papillus of the inner circle and the other branches to the papillae, or papillus, of the outer circle. Between the lips can be seen the outlines of the upper pharyngeal cuticularized supports.

Examination of the stomal region of *A. hamatospicula* shows that a true stoma is represented only by a few muscle bands, the esophageal tissue extending into the base of each lip. The esophagus is divided into 3 distinct regions—a cylindrical corpus, an isthmus, and a valvulated bulb. The corpus is composed of a heavily sclerotized procorpus, or "pharynx" region, and a typically triradiate and less heavily sclerotized metacorpus. The isthmus has a very thin lining but the bulb proper contains a complicated and heavily cuticularized set of valves.

The sclerotized areas of the procorpus (Fig. 1, C) are divided into 4 groups. The most anterior group is composed of 3 units (Fig. 1, D), each of which supports the upper margin of the triradiate lumen and extends down the sides of each muscular wall for a short distance. The major length of the pharynx is supported by 3 pairs of rod-like thickenings placed close to the center of the triradiate lumen (Fig. 1, E). At the base of the pharynx are 2 sets of cuticularized structures: one composed of 3 pairs of short rods which serve as continuations of the more anterior ridges, and the other composed of 3 crescentic thickenings marking the external junction of the pro- and metacorpus. The metacorpus is supported by sclerotized structures of 2 types: one consisting of heavier material grouped lengthwise of the organ near the center of the lumen, and the other consisting of a lining of the "tubes" at the outer ends of the rays of the triradiate canal. These structures show very definite transverse parallel markings. They apparently do not serve as attachment points for the radial muscles as they are internal, not external, thickenings of the esophageal lining.

The triradiate lightly cuticularized lumen of the isthmus opens into the valvulated bulb (Fig. 1, F—valves relaxed). The lumen of the bulb consists of a prevalvular poorly cuticularized region, and of the sclerotized valves—3 in number. The upper portion of each valve is lined with sclerotized material which is emphasized along the inner valve margin. Posterior to this region the flaps are supported by rod-like thickenings of the lining. These rods consist of a smaller basal piece and a larger anterior portion. These rod-like ridges seem to serve as the points of attachment of the muscles controlling the movements of the valves. The thinly cuticularized lining of each of the posterior flaps of the valve extends slightly into the dilated lumen of the anterior end of the intestine.

The accessory piece (Fig. 1, G), a portion of the spicule pouch embedded in the dorsal wall of the cloaca, is a triangular scoop-shaped structure of lightly sclerotized material. The margins of the sides of the triangle are heavily reinforced for support.

The spicules (Fig. 1, H) are flexible tubes of cuticularized material showing the notched distal end characteristic of this particular species. The walls of the basal region (sometimes extending almost half of the length of the spicule) are not solid, but appear to consist of a mosaic of small columnar pieces of heavier material embedded in a matrix of a less dense nature. This gives a distinctly pebbled appearance to the basal portion of each spicule. The tip of each spicule is

covered by a cap-like easily detachable coating of very thin material. This cap may be present at all times but is too transparent to be seen except when the spicule is exerted.

Cuticularization of these various structures appears earliest in the anterior flaps of the esophageal valves. The immature worms of both sexes seem to have completed the sclerotization process, at least as far as the digestive system is concerned, before the accessory reproductive structures begin their final development.

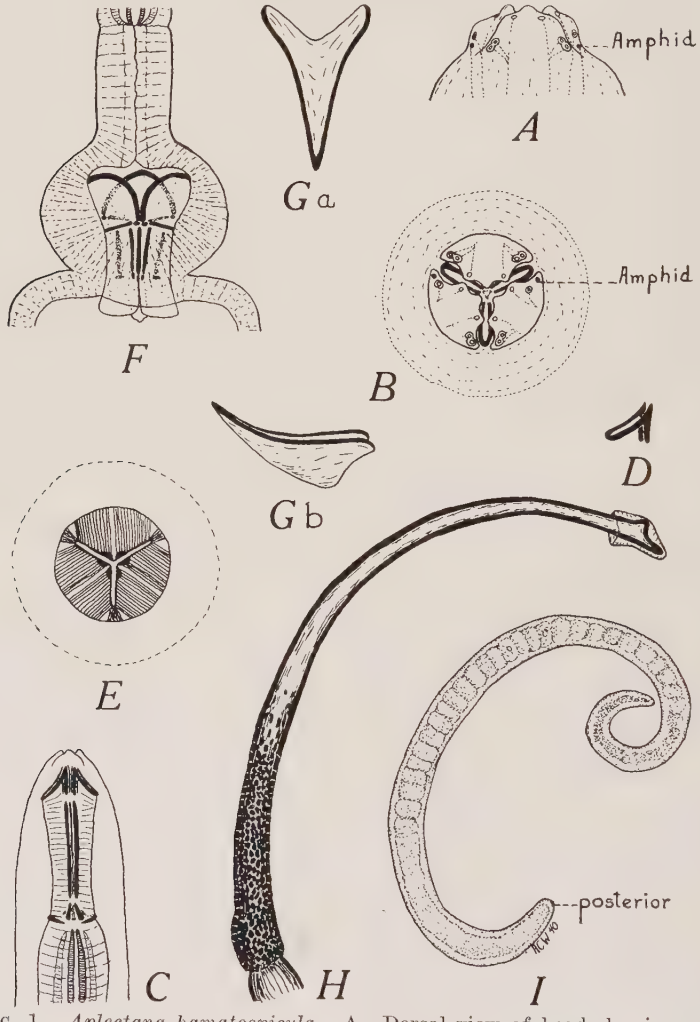

FIG. 1. *Aplectana hamatospicula.* A—Dorsal view of head showing cephalic papillae and amphids, with the innervation of each. B—*En face* view of the head showing the cephalic structures. C—Dorsal view of the pharyngeal and anterior esophageal region, showing the sclerotized areas. D—Detail of one of the sclerotized elements supporting the anterior end of the pharyngeal lumen. E—Cross section through the mid region of the pharynx, showing the position of the strengthening rods and of the pharyngeal muscles. F—Ventral view of the esophageal bulb, showing the details of the valvular apparatus. G—Gubernaculum from ventral (a) and lateral (b) aspects. H—Spicule, side view. I—Newly hatched larva from culture.

First-stage larvae (Fig. 1, I), obtained by culturing eggs shed by mature females, show no stage of internal cuticularization, but do show cellular organization as being more advanced in the posterior esophageal and anterior intestinal region. Stages between such early larvae and the immature adults have not as yet been found, either in cultures or in the amphibian host.

The study of the cephalic and esophageal structures of *Aplectana hamatospicula* substantiates the placing of the genus to which it belongs in the Ascaridoidea instead of in the Oxyuroidea. Examination of four other species of *Aplectana* (including *A. acuminata*), and of four species of *Oxysomatium* (*O. americana, O. longicaudata, O. macintoshii,* and *O. ranae*) show them to be congeneric on the basis of their cephalic and esophageal structures. *Aplectana acuminata* has been designated as the type species of the genus *Aplectana.* The genus *Oxysomatium* (*O. brevicaudatum* is the type species) antedates *Aplectana.* Since some species of each genus are definitely congeneric, including the type of *Aplectana,* it seems to indicate that *Aplectana* must fall as a synonym of *Oxysomatium.* Before this synonymy can definitely be established, however, it must be shown that these various congeneric species are also congeneric with the type species of *Oxysomatium.* If the two types are congeneric, then *Aplectana* must fall; if not, then all of the species of the two genera must be examined to determine their actual relationships. Some species have already been transferred into still other genera because of failing to fit into either of the original generic diagnoses, and probably others will similarly be found to be misplaced. Unfortunately well authenticated specimens of *O. brevicaudatum* (a European form) are not available for study, and the lack of critical details in the earlier descriptions still leaves the problem in abeyance.

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***Aphelenchoides megadorus,* a new species of Tylenchoidea (Nematoda).**

Merlin W. Allen, U. S. Bureau of Plant Industry, Salt Lake City, Utah.

This species of *Aphelenchoides* was rather common in 2 of 15 samples of desert soil taken near the roots of shadscale *Atriplex confertifolia* (Torr. and Frem.) S. Wats., west of Utah Lake, Utah. It is especially distinctive because of the extremely strong spear, heavily sclerotized pharyngeal walls and supporting structures of the head, and the unusual guiding apparatus of the spear. It is possible that, being forced to feed on the hard woody tissues of desert plants, the species has developed an extreme specialization of the mouthparts.

Aphelenchoides megadorus, new species

Total length – 0.5 mm; $\alpha = 31$; $\beta = 8.3$; $\gamma = 22.7$; $V^{-30}72^{8}$. Body nearly cylindroid. Striations of cuticle moderately coarse, those opposite base of spear being 1.1 μ wide and those at middle of body 1.7 μ. Wing areas marked by 3 longitudinal lines occupying one-fifth the body width. Head definitely set off by constriction, one-half as wide as base of neck. Lip region (as seen *en face*) divided into 6 sectors, Amphidial apertures minute, located slightly dorsad on the lateral lips. Papillae 4, very obscure, located near the middle of the submedian lips. Cephalic framework, presumably a development of the cheilorhabdions, heavily sclerotized. Anterior

portion of this framework (*en face*) having 6 subsurface radii, each dividing near base of head (Fig. 1, D). Basal portion of this framework therefore having 6 double radii lying in basal plane of head (Fig. 1, B). Framework immediately surrounding the vestibule hexagonal in cross-section. Walls of pharynx heavily sclerotized, triquetrous in cross-section. Spear 17 μ in length, massive, with large knobs. Anterior portion of spear very slender and supported in spacious pharynx by a supplementary sclerotized sheath or guiding apparatus.

Esophagus at first about one-half as wide as the neck then tapering to a slender tube. A distinct group of cells surrounding esophagus just anterior to bulb; its nature not determined. Bulb glandular anteriorly, slightly elongate, three-fifths the body width. Dorsal esophageal gland opening into lumen of bulb anterior to valve. Submedian esophageal glands opening into lumen of bulb posterior to valve. Intestine beginning as slender transparent tube in which there are no granules until after

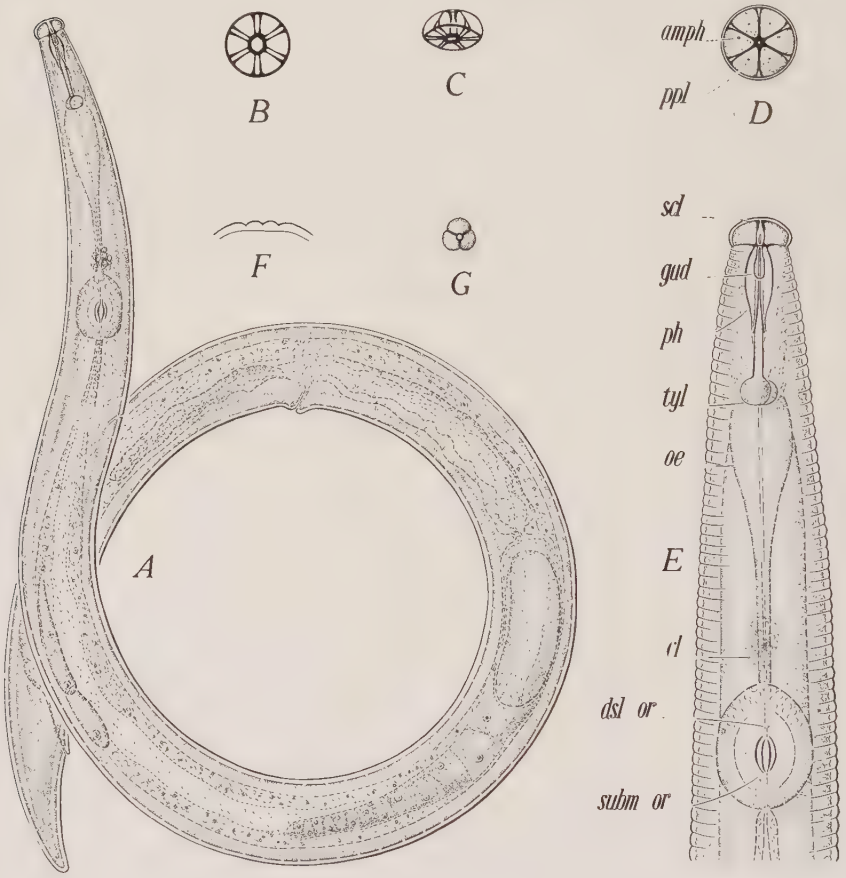

FIG. 1. *Aphelenchoides megadorus*. A—Female; ×730. B—Basal plate of cephalic framework; ×1350. C—Cephalic framework; ×1350. D—*En face* view of head; *amph*, amphid opening; *ppl*, one of the 4 submedian papillae; ×1350. E—Neck region; *scl*, cephalic framework; *gud*, guiding sheath of spear; *ph*, heavily sclerotized pharyngeal walls; *tyl*, knobs of spear; *oe*, esophagus; *cl*, group of cells surrounding esophagus; *dsl or*, dorsal gland orifice; *subm or*, submedian gland orifice; ×1350. F—Wing area in cross section; ×1350. G—Knobs of spear in cross section; ×1350.

it passes through the nerve ring. Nerve ring one body width behind bulb. Excretory pore opposite nerve ring. Dorsal esophageal gland extending 3 to 5 body widths behind bulb. Submedian esophageal glands extending 2 to 4 body widths behind bulb, one on each side of dorsal gland. Cells of intestine 16, polynucleate. Ovary short, outstretched, having about 16 oöcytes arranged in single file. Eggs one-half as wide as body and 3½ times as long as wide. Uterus 3 body widths in length, set off from ovary by constriction. Vulva with slightly elevated lips. Posterior uterine branch 2 to 3 times as long as body width. Rectum length about equal to anal body diameter. Phasmids and caudal pores not seen. Tail blunt and rounded, without mucronate process. Male unknown and gravid females without spermatozoa.

Diagnosis.—Aphelenchoides differing from other members of the genus by the following characters: Heavily sclerotized triquetrous pharyngeal walls, sclerotized spear guiding apparatus, exceptionally large knobs of the spear, enlarged anterior portion of esophagus, and absence of a mucronate process of the tail.

Type Locality.—Desert 2 miles west of Utah Lake, above the abandoned Mosida irrigation project, Utah.

The knobs and the shaft of the spear were found to be soluble in artificial gastric juice (pepsin 4 grams, hydrochloric acid 10 cc, water 900 cc). The anterior portion of the spear, the guiding sheath, sclerotized pharynx and the supporting framework of the head were not dissolved. The parts not dissolved were observed to be molted with the cuticle.

Several specimens of *Aphelenchoides megadorus* were infested with a sporozoan parasite, probably *Duboscqia penetrans* Thorne (1940, Proc. Helminth. Soc. Wash. 7(1): 51–53).

Description based on living and fixed specimens. Illustrations from fixed specimens mounted in glycerine.

Notes on the life history of the root-knot nematode, *Heterodera marioni*. J. R. Christie and Grace Sherman Cobb, U. S. Horticultural Station, Beltsville, Md.

Recently we have had occasion to reexamine the literature pertaining to the life history of *Heterodera marioni* (Cornu, 1879) Goodey, 1932. Although statements regarding habits and behavior are scattered through the numerous publications dealing with this important plant parasite, the only comprehensive account of its life history is that given by Nagakura (1930, Japanese Jour. Zool. 3(3): 95–160). On certain details, notably the time of molting, neither Nagakura nor other writers seem to be very explicit and we decided to restudy certain phases of the life cycle. The purpose of this paper is to record briefly some of the results of this study.

The molts.—According to Nagakura, the first molt takes place within the egg shell and gives rise to what he terms the "first" larva. With regard to the second molt Nagakura writes, "Nach dem Freileben von unbestimmter Dauer, dringt die erste Larve in eine Pflanzenwurzel hinein und tritt in den Ruhezustand ein. Nach kurzer Zeit macht sie die zweite Häutung und wird dicker." It would appear from this statement that a larva is supposed to molt soon after entering a root and that the change from a cylindrical to a saccate form takes place, for the most part, after this second molt.

Nagakura then goes on to point out that in the male the third molt, which terminates the "second" stage, is followed by metamorphosis and as soon as metamorphosis is completed the male undergoes its fourth and final molt. Nagakura sets forth this latter fact as follows: "Sobald die Entwicklung aller Organe vollendet ist, macht die dritte Larve eine Häutung und wird zum Erwachsenen."

Nagakura further points out that the female molts a third time at approximately the same stage of development as the male but undergoes no corresponding change in form. During this molt the caudal spike is lost and at about this time the vulva is formed. The fourth and final molt of the female is accounted for by the following statement: "Die dritte Larve macht eine Häutung und wird zum Erwachsenen, nachdem das Tier ein bedeutendes Dickenwachstum durchgemacht hat."

It is customary to regard the first molt as terminating the first and initiating the second larval stage, the second molt as terminating the second and initiating the third larval stage, etc. As we will follow this custom it should be borne in mind that, with reference to molting, our second stage corresponds with Nagakura's

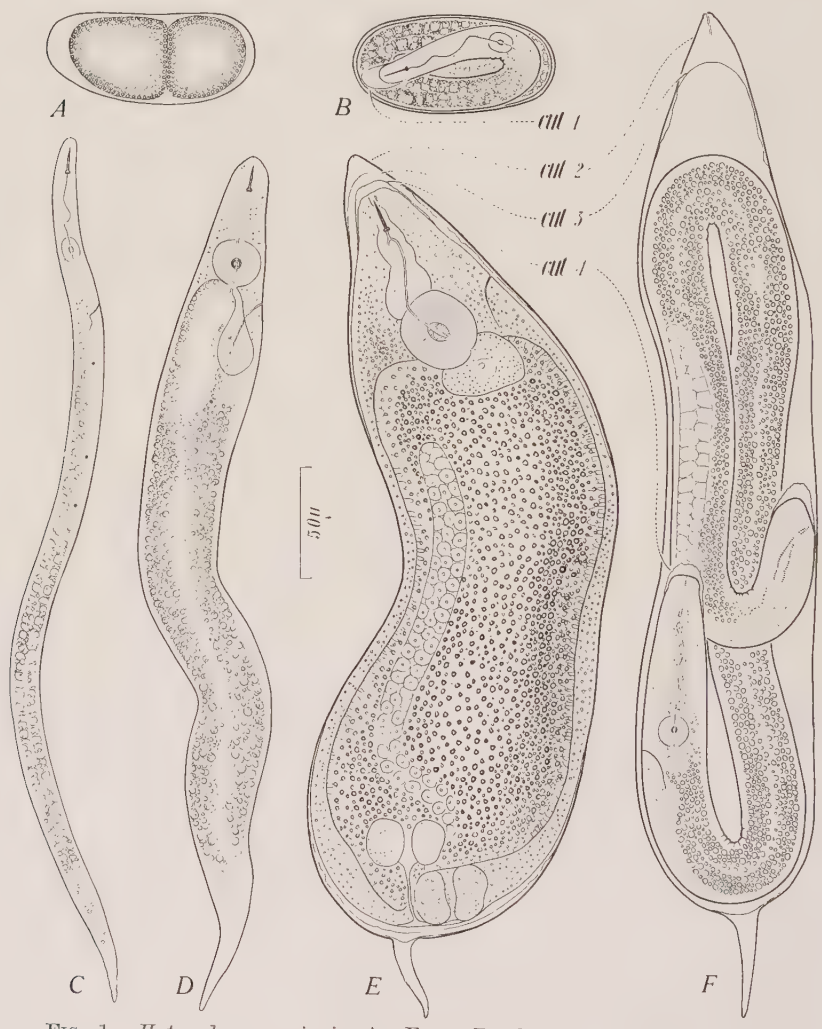

Fig. 1. *Heterodera marioni*. A—Egg. B—Ovic larva showing first molt. C—Preparasitic, second-stage larva. D—Parasitic, second-stage larva. E—Female showing second, third and fourth molts. F—Metamorphosing male showing second, third and fourth molts. *cut 1*, first molted cuticle; *cut 2*, second molted cuticle; *cut 3*, third molted cuticle; *cut 4*, fourth molted cuticle.

''first'' stage and so on. In general the results of our study have verified the facts set forth in Nagakura's excellent monograph but we believe he errs on some points and that others need further elucidation.

The specimens used in this study were from a population of *Heterodera marioni* that had been maintained on tomato plants in the greenhouse for a year or two.

The ovic larva molts for the first time before it has attained its maximum length and while it is still relatively inactive. Molting specimens removed from the egg move sluggishly. If movement is active the larva has already completed this molt. It is not difficult to see the separated cuticle protruding beyond the head of a larva that is still within the egg shell (Fig. 1, B).

The sac within which the male undergoes its metamorphosis is composed of 2 layers, a comparatively thick outer layer and a much thinner inner layer. These are the second and third molted cuticles, respectively (Fig. 1, F). The duplex nature of this sac first came to our attention when we saw a newly metamorphosed male that, in some manner, had been removed from the outer layer but was still encompassed by the inner layer. This inner cuticle was so thin as to be almost invisible yet was apparently rather tough. In agreement with Nagakura, we found that a male molts a fourth time immediately on completing its metamorphosis and while it is still within the sac formed by the second and third cuticles (Fig. 1, F).

The molts of the female, at the time the caudal spike is lost, correspond to the molts of the male at the time of metamorphosis. Two cuticles are simultaneously detached and loosen about the body of the female, then, very shortly thereafter, another cuticle is detached. These cuticles represent the second, third, and fourth molts, respectively (Fig. 1, E).

No molt occurs in either sex from the time a larva escapes from the egg until the simultaneous loosening of the second and third cuticles followed shortly by the fourth. The entire first stage is passed within the egg shell. The second stage begins within the egg, includes the free-living, preparasitic period, and the first part of parasitic development. There is no third stage in either sex. In the male there is a distinct fourth stage during which metamorphosis takes place. In the female there appears to be a very brief interval between the detachment of the second and third cuticles and the detachment of the fourth cuticle, hence, strictly speaking, there is a fourth larval stage. However, these last 3 molts are so nearly simultaneous that the fourth stage is largely theoretical.

Growth.—There appears to be no way of distinguishing sex until the end of the second stage. By this time the female has increased considerably in width though very little in length. The male likewise has increased in width though frequently not so much as the female. After completing the last 3 molts the adult female continues to grow and there is a very great all-round increase in size.

The Stylet.—The stylet of Nagakura's ''first'' larva (i.e., the preparasitic larva) was 20 to 25 μ long. The stylet of his ''second'' larva (i.e., after the alleged second molt) was only 9.2 μ long. In the male the stylet of his ''third'' larva and that of the adult were 20 μ long and in the female the stylet of his ''third'' larva was 13.3 μ long and that of the adult 17.5 to 25 μ long.

It is sometimes difficult to measure the stylet for the tip is not always easy to see. In some of the specimens that we studied the stylet was protruding slightly thus making it possible to locate the tip very exactly. In other specimens the tip was located as nearly as possible. In our material the stylet of the preparasitic larva varied from 13 to 14 μ in length.

So far as we could determine there is no change in the stylet during the second stage (which includes Nagakura's ''first'' and ''second'' stages) until the approach of the two simultaneous molts. Just prior to molting, however, the basal portion of the stylet becomes indistinct and the lining of the anterior portion is

shed with the second cuticle. We saw no remnant of the stylet on the third or fourth cuticles in either sex and the stylet is not completely reformed until after the final molt.

In our specimens the stylet of the adult female varied in length from 14 to 17 μ and the stylet of the adult male from 23 to 25 μ. In other words the stylet of the adult female was only slightly longer, though noticeably heavier, than that of the preparasitic larva while the stylet of the adult male was about twice as long as, and very much heavier than, that of the preparasitic larva.

The size of the stylet may, perhaps, differ in different populations, and Nagakura's measurements may very well be correct except the one that applies to his "second" larva, which was probably derived from molting specimens.

Note on staining nematodes in root tissue. C. W. McBeth, A. L. Taylor, and A. L. Smith, U. S. Department of Agriculture, Bureau of Plant Industry.

Goodey (1937, Jour. Helminth. **15**(3): 137–144) describes a method of staining nematodes in plant tissue using cotton blue or acid fuchsin stain in lactophenol. By this method the plant tissue is first boiled in the stain solution for one minute, then washed in clear water and dehydrated in the usual series of graded alcohols, and finally cleared in clove oil. Very good results can be realized by this method only if the tissue is dehydrated gradually, otherwise it will be shrunken and the nematodes obscured. Dehydration also calls for handling the tissue several times and requires considerable alcohol.

Due to the need for staining and counting nematodes from hundreds of roots a simplified method was devised which eliminates the necessity of dehydrating the tissue. Goodey's formula for staining was used, as follows:

Phenol, pure crystals, 20 grams
Lactic acid (sp. gr. 1.31), 20 grams
Glycerine (sp. gr., 1.25) 40 grams
Distilled water 20 cc
Acid fuchsin or cotton blue, 5 cc (1 gram to 100 cc H_2O)

First wash the roots thoroughly in water to remove soil particles and boil in staining solution for one minute. Wash in tap water to remove excess stain and place in clear lactophenol solution until cleared. The same lactophenol formula is used for clearing that is used for staining except that no stain is added. Clearing requires from one to several days, depending on the type of roots and intensity of stain.

After the tissue has cleared, it can be examined in a Syracuse watch glass or Petri dish covered with clear lactophenol or glycerine. If permanent mounts are desired, the material can be mounted directly in glycerine.

In some cases, better results are obtained by using a weaker stain concentration, ½ cc of stain solution in 100 cc of clear lactophenol. If this weaker concentration is used, the material must be boiled for 2 minutes in clear lactophenol and 1 minute in the stain, or for 2 minutes in the stain.

A practical method of using methyl bromide as a nematocide in the field. A. L. Taylor and C. W. McBeth, U. S. Bureau of Plant Industry.

Preliminary experiments previously reported (Proc. Helminth. Soc. Wash. **7**(2): 94–96. July, 1940) indicated that methyl bromide has some very valuable characteristics as a soil nematocide for use against the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. In these experiments the chemical was used on soil confined in a fumigation box and was also introduced into buried drain tile lines.

Further experiments with soil in a fumigation chamber indicated that all

root-knot nematodes and all free-living nematodes in sandy loam soil could be killed by the use of 300 cc of methyl bromide gas (at 25° C. and atmospheric pressure) per cubic foot of soil. Time of exposure to the gas was 24 hours. This corresponds to slightly less than 0.75 cc of liquid methyl bromide (at 0° C.) to the cubic foot of soil.

As it is often desirable to control root-knot in soil in the field, in greenhouse beds or benches, in seed beds or in ornamental flower beds, a method of using methyl bromide was devised and tested as follows:

A strip of Norfolk sandy loam soil known to be heavily infested with root-knot nematodes was plowed and leveled. Six plots each 8 feet wide and 25 feet long were prepared. Gas-tight covers were placed over 3 of these. The covers were made by gluing three 4-foot strips of glue-coated kraft paper together to form a sheet 10 feet wide and 27 feet long. The paper over the plot was held about 3 inches above the soil surface by small mounds of soil. The extra foot of paper on each margin was buried.

A tube attached to the methyl bromide cylinder was inserted through a small hole in the paper cover and 1½ lbs. (393 cc) of methyl bromide discharged over each of the covered plots. Since this amount was the full content of the cylinder, all that was required was to open the cylinder valve and allow the chemical to be forced out by its self-generated pressure. (Methyl bromide boils at 4.6° C. and is therefore a gas at higher temperatures.) When the cylinder was empty—which required less than 2 minutes—the tube was withdrawn and the small hole covered with a patch of paper. Since the object of the experiment was to test a method, all the covered plots received identical treatment. At the time the chemical was applied, the soil temperature was 25° C. and the soil moisture 2.7 per cent.

The paper covers were removed after 48 hours. Soil samples taken from the treated plots at this time contained no living (but many dead) free-living nematodes. Similar samples from the adjacent control plots contained many living nematodes.

All the plots were immediately planted to lima beans which were allowed to grow for 6 weeks, then removed and examined microscopically for root-knot nematodes. Plants from the 3 control plots were heavily attacked by root-knot nematodes, 97 per cent, 94 per cent and 100 per cent of the roots being infested, while those from the 3 treated plots contained no root-knot nematodes at all.

DISCUSSION

The method described above appears to be a convenient, simple and effective way of applying methyl bromide to soil. It has an advantage over the fumigation-box method in that the soil can be treated without removal from the place where it is to be used. In the present experiment 200 square feet of soil in each plot were treated to a depth of at least 1 foot and probably more. If the depth of the treatment be estimated at 1 foot, 200 cubic feet or 7.4 cubic yards of soil were freed of root-knot. Material required was 1.97 cc of methyl bromide per cubic foot. Time was less than 1 hour per plot, most of which was occupied in preparing and placing the paper cover. There appears to be no reason why other gas-tight material, such as rubberized canvas, could not be used for covers in place of glue-coated kraft paper, which is efficient but is easily damaged.

The above experiment gives no information on treating areas greater than 200 square feet under one cover with the chemical applied at only one point, nor does it indicate how much less than 1.97 cc per square foot of surface might be used. No plant-parasitic nematodes other than *H. marioni* were present in the soil used. However, since the free-living nematodes were killed by the chemical, it seems probable that other parasitic species could also be controlled.

CONCLUSION

Methyl bromide is an effective soil nematocide for use in the field with sandy loam soil at 25° C. when applied by simply introducing it under a gas-tight cover over the soil to be treated. Using a glue-coated kraft paper cover, 1½ lbs. of methyl bromide completely controlled both root-knot and free-living nematodes in 200 square feet of sandy loam soil in the field.

A summary of the Physalopterinae (Nematoda) of North America.[1] BANNER BILL MORGAN, University of Wisconsin, Department of Zoology.

Recent research shows that the genus *Physaloptera* is one of the largest and taxonomically one of the most unwieldy of the genera of parasitic nematodes. As this group became larger and larger, species determination has become difficult. Inadequate descriptions of certain species, lack of organized keys, and the widely scattered literature have complicated the situation. Consequently, many synonyms have been created. Schulz (1927, Samml. Helminth. Arb. 287–312) divided *Physaloptera* into 3 genera based primarily on the dentition of the pseudolabia. The present writer recognizes as valid 4 genera, as follows: *Physaloptera* Rudolphi, 1819, *Abbreviata* (Travassos, 1920) Schulz, 1927, *Skrjabinoptera* Schulz, 1927, and *Pseudophysaloptera* Baylis, 1934.

Positive identification of species can be made only on characters possessed by both male and female specimens. The characters of value for species determination in the Physalopterinae are as follows, in the order of their importance: (1) Dentition (generic value), (2) number of uteri, (3) mode of origin of uteri, (4) number of male ventral papillae, (5) arrangement of male ventral papillae, (6) shape of spicules, (7) length of spicules, and (8) position of vulva.

Certain characters cannot be used for specific determination because of the wide variations within species: (1) Posterior sheath, (2) size and height of teeth, (3) length of esophagus, (4) position of excretory pore and cervical papillae, (5) size of eggs, (6) shape of bursa, (7) bursal markings, and (8) shape of seminal receptacle.

From material collected during the past 3 years, material examined at the U. S. National Museum, and from descriptions given in the literature, the following species of Physalopterinae have been definitely established as occurring in North America. The species whose names are marked with an asterisk (*) have not been examined by the writer. All of the parasites were from the stomach unless otherwise noted. Of more than 150 species of Physalopterinae that have been proposed, many of which are synonyms, less than 25 are known to occur in North America. Of this small number, only 8 species are known to be at all common.

Abbreviata:

1. *A. ranae* (Walton, 1931). (Larval form)
   Rana catesbeiana (Bullfrog)
   R. sphenocephala (Leopard Frog)
   Bufo woodhousii (Toad)
2. *A. terrapenis* (Hill, 1940).
   Terrapene ornata (Western Box Turtle)
3. *A. varani* (Parona, 1889). Syn. *A. quadrovaria* (Leiper, 1908).
   Sceloporus undulatus (Eastern Swift Lizard)

Skrjabinoptera:

1. *S. phrynosoma* (Ortlepp, 1922).
   Phrynosoma cornutum (Texas Horned Lizard)
   P. d. douglassi (Douglass Horned Lizard)
   P. d. hernandezi (Hernandez Horned Lizard)

[1] This investigation was aided by a grant from the Wisconsin Alumni Research Foundation.

P. b. blainvilli (Blainville Horned Lizard)
P. solare (Regal Horned Lizard)
P. platyrhinos (Pacific or Smooth Horned Lizard)
Sceloporus spinosus (Spiny Tree Lizard)
S. torquatus (Collared Tree Lizard)

Physaloptera:

1. *P. abjecta* Leidy, 1856. Syn. *P. variegata* Reiber, Byrd, and Parker, 1940.
   Coluber f. flagellum (Coach Whip Snake)
   C. c. constrictor (Black Snake)
   C. c. flaviventris (Western or Blue Racer)
   Lampropeltis getula nigra (King Snake)
   Thamnophis sirtalis (Garter Snake)
2. *P. acuticauda* Molin, 1860. Syn. *P. truncata* Schneider, 1866; *P. quadridentata* Walton, 1927.
   Buteo platypterus (Broad-winged Hawk)
   B. borealis (Red-tailed Hawk)
   B. lineatus (Red-shouldered Hawk)
   Geococcyx californianus (Road Runner)
3. *P. alata* Rudolphi, 1819. Syn. *Spiroptera physalura* Dujardin, 1845.
   Accipiter velox (Sharp-shinned Hawk)
   A. cooperi (Cooper's Hawk)
4. *P. bispiculata* Vaz and Pereira, 1935.
   Sigmodon hispidis (Cotton Rat)
   Cynomys ludovicianus (Prairie-dog)
   Ondatra rivalicia (Louisiana Muskrat)
5. *P. felidis* Ackert, 1936.
   Felis domestica (Cat)
   Canis familiaris (Dog)
   C. latrans (Coyote)
   Vulpes fulva (Silver Fox)
6. *P. limbata* Leidy, 1856.
   Scalopus a. aquaticus (Eastern Mole)
   S. a. machrinus (Prairie Mole)
   S. a. machrinoides (Missouri Valley Mole)
   Parascalops breweri (Hairy-tailed Mole)
7. *P. maxillaris* Molin, 1860. Syn. *P. semilanceolata* Molin, 1860; *P. mephites* Solanet, 1909; *P. mydai* Baylis, 1926.
   Spilogale putorius (Spotted Skunk)
   S. g. gracilis (Canyon Spotted Skunk)
   S. g. saxatilis (Great Basin Spotted Skunk)
   S. interrupta (Prairie Spotted Skunk)
   Mephitis mephitis (Canada Skunk)
   M. m. mesomelas (Louisiana Skunk)
   M. m. nigra (Eastern Skunk)
   M. m. avia (Illinois Skunk)
   M. elongata (Florida Skunk)
   M. o. occidentalis (California Skunk)
   M. hudsonica (Northern Plains Skunk)
   M. macroura milleri (Northern Hooded Skunk)
   Conepatus mesoleucus venaticus (Arizona Hog-nosed Skunk)
   Taxidea taxus taxus (Common Badger)
   T. t. berlandieri (Mexican Badger)
   T. t. neglecta (Western Badger)
   Mustela vison mink (Common Mink)
   M. v. vulgivaga (Southern Mink)
   Procyon lotor lotor (Eastern Raccoon)
   P. l. elucus (Florida Raccoon)
8. *P. mexicana* Caballero, 1937.
   Falconidae (Falcons)
9. *P. muris-brasiliensis* Diesing, 1861. Syn. *Spiroptera bilabiata* Molin, 1860; *P. circularis* Linstow, 1897; *P. scuiri* Parona, 1898; *P. ruwenzorii* Parona, 1907.
   Sigmodon h. hispidis (Cotton Rat)
   Rattus norvegicus (Common Rat)
10. *P. obtusissima* Molin, 1860. Syn. *P. monodens* Molin, 1860; *P. squamatae* Harwood, 1932.
    Leiolopisma laterale (Brown Lizard)

Agkistroden mokasen (Water Moccasin)
Crotales confluenta oreganus (Pacific Rattle Snake)
Coluber constrictor mormon (Western or Blue Racer)
C. c. constrictor (Black Snake)
C. c. flaviventris (Western or Blue Racer)
Heterodon contortrix (Puffing Adder)

11. *P. praeputialis* Linstow, 1889. Syn. *Chlamydonema felineus* Hegt, 1910; *C. praeputialis* of various authors; *C. praeputiale* Yorke and Maplestone, 1926.
Felis domestica (Cat)
F. p. pardalis (Ocelot)
F. couguar (Mountain Lion)
Lynx canadensis (Lynx or Bobcat)
L. rufus baileyi (Bailey Bobcat)
L. rufus texensis (Texas Bobcat)
L. uinta (Mountain Bobcat)
Canis familiaris (Dog)
Urocyon c. cinereoargenteus (Eastern Gray Fox)
U. c. borealis (Northern Gray Fox)
Vulpes fulva (Silver Fox)

12. *P. rara* Hall and Wigdor, 1918. Syn. *P. cerdocyona* Sprehn, 1932; *P. clausa* of Caballero, 1937.
Canis familiaris (Dog)
C. latrans (Coyote)
C. nubilus (Timber Wolf)
C. n. nebracensis (Prairie Wolf)
C. ochropus (Valley Coyote)
Urocyon c. cinereoargenteus (Eastern Gray Fox)
U. c. scotti (Arizona Gray Fox)
U. c. floridanus (Florida Gray Fox)
U. c. californicus (California Gray Fox)
Vulpes fulva (Silver Fox and Eastern Red Fox)
V. regalis (Northern Plains Red Fox)
Procyon l. lotor (Eastern Raccoon)

13. *P. retusa* Rudolph, 1819. Syn. *Spiroptera retusa* Dujardin, 1845; *P. largarda* Sprehn, 1932.
Alligator mississippiensis (Alligator)*
Sceloporus undulatus (Eastern Swift Lizard)
S. g. graciosus (Swift Lizard)

14. *P. spinicauda** McLeod, 1933.
Citellus franklini (Franklin Ground Squirrel)
C. t. tridecemlineatus (Thirteen-striped Ground Squirrel)

15. *P. torquata* Leidy, 1886.
Taxidae taxus taxus (Common Badger)
T. t. neglecta (Western Badger)
Procyon l. lotor (Eastern Raccoon)

16. *P. turgida* Rudolphi, 1819. Syn. *Spiroptera turgida* Dujardin, 1845; *Turgida turgida* Travassos, 1920; *P. ackerti* Hill, 1939.
Didelphis v. virginiana (Common Opossum)
D. v. pigra (Florida Opossum)
D. mesamericana texensis (Texas Opossum)

Pseudophysaloptera:

1. *P. soricina* Baylis, 1934.
Sorex p. personatus (Masked Shrew)

Physaloptera papillotruncata Molin, 1860, identified by Canavan (1931, Parasitol. 23(2): 196–229) from the Badger (*Taxidae taxus*) and *P. turgida* Rudolphi, 1819, identified by Leigh (1940, Ill. Nat. Hist. Bul. 21(5): 185–194) from the Canada Skunk (*Mephitis mephitis*) and Eastern Raccoon (*Procyon lotor*) has not been examined by the writer. These authors did not state definitely the characters on which their identifications were based. Until more evidence is presented or the material re-examined, these determinations must remain doubtful.

Report of the Brayton H. Ransom Memorial Trust Fund

December 31, 1940

The trustees met on December 19, 1940, and voted an award of $35.00 for 1940, to the Proceedings of the Helminthological Society.

The status of the Fund, since the previous statement in the Proceedings of the Helminthological Society, January, 1940, is as follows:

On Loan	$1350.00
Balance on Hand, December 31, 1939	51.68
Receipts:	
Bank interest to Jan. 1, 1940	.51
Interest on loan to Jan. 12	27.00
Bank interest to July 1	.70
Interest on loan to July 12	27.00
Total Receipts	$ 106.89
Disbursements:	
For safe deposit box:	
Balance of 1939 rent	1.85
1940 rent	3.85
Award to Helminthological Society	35.00
Total Disbursements	$ 40.70
Balance on Hand, December 31, 1940	66.19
	$ 106.89

Eloise B. Cram,
Secretary-Treasurer

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